IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants: Frank A. Skraly and Martha Sholl

Serial No.: 09/909,574 Art Unit: 1652

Filed: July 20, 2001 Examiner: Yong D. Pak

For: *PRODUCTION OF POLYHYDROXYALKANOATES FROM POLYOLS*

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Commissioner for Patents
P.O. Box 1450
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REPLY BRIEF

Sir:

This is a Reply Brief to the Examiner's Answer mailed on December 28, 2007. A Notice of Appeal was filed on June 22, 2007. Submitted with this Reply Brief is a Request for Oral Hearing. The Commissioner is hereby authorized to charge $1,030.00, the fee for filing a Request for Oral Hearing for a large entity to Deposit Account No. 50-3129.

It is believed that no additional fee is required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issue presented on appeal is:

whether claims 1-4 and 6-10 are non-obvious as required by 35 U.S.C. § 103(a) over Skraly, Polyhydroxyalkanoates Produced by Recombinant E. coli, Poster Engineering Foundation Conference: *Metabolic Engineering*, 1998 ("Skraly"), Madison, et al. Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic. *Microbiol. Mol. Biol. Rev.* 63(1):21-53 (1999) ("Madison") and BRENDA database ("Brenda").

(7) ARGUMENTS

Appellants affirm all of the arguments made in the Appeal Brief.

Claim 1

Claim 1 is Nonobvious in view of the Prior Art

The prior art does not disclose or suggest the claimed method, alone or in combination. Claim 1 requires in addition to the PHA biosynthestic enzymes, that the organism be genetically engineered to express enzymes selected from the group consisting of diol oxidoreductase and aldehyde dehydrogenase. Skraly does not disclose a method that employs a combination of the enzymes recited in claim 1. The Examiner alleged that Skraly discloses a method for producing PHA from 1,3-propanediol using recombinant *E. coli* expressing PHA synthase and diol oxidoreductase, wherein the diol is oxidized to its corresponding aldehyde and then converted to its corresponding hydroxyalkanoate monomer via an aldehyde dehydrogenase and CoA transferase (citing pages 8-9 of Skraly). The Examiner is correct about the sequence of reactions; however what is relevant to the claimed method is not only the sequence of reactions,

but also the enzymes that are employed. Skraly (page 9) discloses that glycerol dehydratase and 1,3-propanediol (PD) oxidoreductase are found in many organisms, and that these genes have been imported into transgenic *E. coli*, enabling them to synthesize PHB-co-3HP from glycerol and PHB-co-3HV from 1,2-propanediol with or without added glucose. As disclosed in Skraly (see page 10), the studies involved providing the *E. coli* strain MBX820 with the *Klebsiella pneumonia* glycerol dehydratase (and therefore mandatory addition of Coenzyme B-12) and 1,3-PD oxidoreductase genes as well as the 4-hydroxybutyryl-CoA transferase gene from *Clostridium culverin*. PHB-co-HV was more difficult to synthesize (from propane-1,2-diol) since an additional thiolase was required.

This is different from the claimed method, which requires that the organisms be genetically engineered to express enzymes selected from the group consisting of diol oxidoreductase and aldehyde dehydrogenase, in addition to the PHA biosynthetic enzymes recited in claim 1. Furthermore, the claimed method does not employ glycerol dehydratase, or the mandatory coenzyme B-12 that would be needed for organisms such as *E. coli* which cannot synthesize coenzyme B-12, or a second thiolase to convert propionyl CoA (from 1,2-propanediol) to 3-hydroxyvaleryl-CoA.

Thus, Applicants omit two elements required by the method disclosed in Skraly for producing PHA from the diol 1,2-propane diol and still arrive at PHA. The MPEP (§2144.04) notes that the omission of an element and retention of its function is an indicia of unobviousness. The Examiner admitted that Skraly does not teach a method of producing PHA from the diols recited on page 4 of the Examiner's Answer (2nd paragraph), using an *E. coli* expressing diol

oxidoreductase and acyl-CoA transferase, acyl-CoA synthetase, β-ketothiolase, acetoacetyl-CoA reductase or PHA synthase. Appellants agree with the Examiner. However, at least for the reasons discussed above, this is not the only difference between the method in Skraly and the claimed method as alleged by the Examiner.

The Examiner relied on Madison (Examiner's Answer, page 4) as evidence to support the level of skill in the art of recombinant organisms expressing all genes necessary to produce PHA's. It appears as though the Examiner is arguing that because the level of skill in the art is high, one can make anything. That is not the case. Appellants agree that the level of skill in the art of *expressing* genes necessary to produce PHA's was high; however, one still had to determine what pathway (and hence what genes) to engineer into the organism of choice, to enable and/or enhance PHA production in a non-PHA producing organism. The Examiner also relied on Madison for disclosing that the molecular mass of PHAs produced varies from 50,000 to 1,000,000 DA and that bacterially produced PHAs have a high molecular weight. The Examiner has mischaracterized the disclosure in Madison and has not considered other disclosure in Madision. Madison (see page 22) states that the molecular mass of **poly-3-hydroxyalkanoates** varies per **poly-3-hydroxyalkanoate** producer but is *generally* on the order of 50,000 to 1,000,000 Da. This is not tantamount to a disclosure that the molecular mass of all polyhydroxyalkanoates is invariably in the order of 50,000 to 1,000,000 Da in every poly-hydroxyalkanoate producer, let alone in genetically engineered organisms producing PHA with the monomer content recited in claim 1. Furthermore, Madison also discloses conditions in which the molecular mass of poly-3-hydroxyalkanoate obtained was less than 50,000 (see

Madison, page 39). Even if disclosure in Madison were limited to the mass range cited by the Examiner, knowledge of the mass range of poly-3-hydroxyalkanoates does not make obvious every and any genetically engineered pathway that produces PHAs in that size range.

The Examiner cited from Appellants' Appeal Brief filed on June 5, 2006, at pages 24-25, where Appellants disclose that the enzymes recited in claim 1 were known in the art as well as techniques to express them in cells (in response to an enablement rejection) and then to Brenda for disclosing several diol reductases that oxidize diols (Examiner's Answer, paragraph bridging pages 4 and 5, and page 5, 2nd paragraph). Again, the Examiner is missing a key point, which is, that Appellants have identified an alternate genetically engineered pathway (i.e. utilizing a novel combination of enzymes) for PHA production. Appellants did not, however, discover nor are they claiming the individual genes in the pathway, which are known. That is all Brenda provides, one of the genes in the pathway. Without identification of the pathway, one of ordinary skill in the art would not have known what enzymes must be engineered by the engineered organism. However, the Examiner alleged (on page 7 of the Examiner's Answer) that this is not persuasive since Appellants also point out that "it was routine to identify enzymes having the appropriate specificity for the cheap substrates which would in turn yield the required monomers".

Appellants respectfully point out that the Examiner has taken Appellants' comments out of context. Appellants stated (paragraph bridging pages 9 and 10 of the Appeal Brief filed on August 22, 2007) that there is a need for a cost effective way to produce PHAs, then further explained that it was in this context that they identified alternative pathways to provide substrates

for the PHA synthase. They accomplished this by starting from cheap available substrates, determining a pathway to produce the necessary monomers that could be utilized by the PHA synthase, and, thus, the enzymes that must be expressed by the engineered organisms. Appellants further stated that **with this knowledge in hand** (i.e. the enzymes that must be expressed) it was then *routine to identify enzymes having the appropriate specificity for the cheap substrate which would in turn yield the required monomers*. One must first identify what enzymes must be expressed before identifying those having appropriate specificity.

The Examiner (page 8 of the Examiner's Answer) relied on Skraly for teaching a method of producing PHA from a cheap diol by genetically engineering an organism expressing a 1,3-PD oxidoreductase and aldehyde dehydrogenase. However, Skraly does not disclose genetically expressing an aldehyde dehydrogenase in *E. coli*; according to the Examiner's notation (Examiner's Answer, page 7, 2nd paragraph) that aldehyde dehydrogenase is endogenous to *E. coli*. Furthermore, the Examiner has completely ignored the requirement for a glycerol dehrydratase and thus coenzyme B-12 disclosed in Skraly, none of which are required by the claimed method. Even if Skraly disclosed a method that employed only engineered 1,3-PD oxidoreductase and endogenous aldehyde dehydrogenase in *E. coli*, Example 4 of the present application compares incorporation of 4HB into a PHA in *E. coli* (which according to the Examiner would endogenously express aldehyde dehydrogenase) genetically engineered to express *dhat* (diol oxidoreductase) only or *dhat* and *aldh* (aldehyde dehydrogenase). The results (shown in Table 1) demonstrate that it is only a when both genes are genetically engineered in *E. coli* (i.e. using the claimed method) that a significant level of 4HB incorporation is achieved-

25.3% of polymer compared to 0.7% with *E. coli* endogenously expressing aldehyde dehydrogenase and genetically engineered to express *dhat* (the method allegedly disclosed by Skraly).

The MPEP notes that rebuttal evidence may include evidence that the claimed invention yields unexpectedly improved properties or properties not present in the prior art and that evidence pertaining to secondary considerations must be taken into account whenever present. (See MPEP § 2145). Thus, even if Skraly disclosed a method that did not require glycerol dehydratase as suggested by the Examiner, Appellants rebut with evidence that the claimed method yields unexpectedly improved properties and would be applicable to a wider range of organism. However, Skraly is clear about the requirement for glycerol dehydratase and coenzyme B-12 (see Skraly, page 11) and there is no motivation for one of ordinary skill in the art to eliminate these two elements disclosed in Skraly. For at least the reasons above, claim 1 is non obvious over a combination of Skraly, Madison, and Brenda.

Claim 2

Claim 2 is Nonobvious In View of the Prior Art

As admitted by the Examiner (Examiner's Answer, page 4, 2nd paragraph) and at least for the reasons set forth above with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of claim 2.

Claim 3

Claim 3 is Nonobvious In View of the Prior Art

As admitted by the Examiner (Examiner's Answer, page 4, 2nd paragraph) and at least for the reasons set forth above with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of claim 3.

However, the Examiner further alleged that Skraly does disclose producing PHAs from 1,5-pentanediol (converted to 5-hydroxyvalerate; Skraly, page 7). However, Skraly also discloses underneath the table on page 7, that in all cases, the organism used was recombinant *E. coli* containing a PHA synthase, and in some cases, 4-hydroxybutyryl-CoA transferase was also used. Example 3 of the present application shows that PHA production (using 1,4-butanediol) in *E. coli* using the Skraly method was 25% of the PHA produced in *E. coli* according to the claimed method (see the specification at least at page 14, lines 1-6). Appellants note that there is no mention of genetically engineering the organism to express an oxidoreductase or an aldehyde dehydrogenase. There is no reason why one of ordinary skill in the art would modify this disclosure in Skraly to include these two enzymes. The Examiner's admission on page 4 of the Examiner's Answer is correct. Skraly does not disclose a method wherein the organism is genetically engineered to express a combination of all of the enzymes recited in the claims. None of Madison or Brenda makes up for this deficiency. Therefore, claim 3 in nonobvious over the prior art.

Claim 4

Claim 4 is Nonobvious In View of the Prior Art

As admitted by the Examiner (Examiner's Answer, page 4, 2nd paragraph) and at least for the reasons set forth above with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of claim 4.

However, the Examiner alleged that Skraly discloses 1,4-butanediol (converted to 4-hydroxybutyrate and a method of engineering an organism to produce PHAs from two different diols using an *E. coli* expressing an endogenous aldehyde dehydrogenase and genetically engineered to express 1,3-propanediol oxidoreductase. As discussed with respect to claim 3, Skraly expressly states what enzymes are used. For reasons set forth with respect to claim 1, Skraly does not disclose the alleged method.The Examiner has provided no reason why one of ordinary skill in the art would modify the disclosure on page 7 of Skraly to add an oxidoreductase. Even if one did, the claimed method produces unexpected results (see unexpected results discussed above with respect to Example 4).

Claim 6

Claim 6 is Nonobvious In View of the Prior Art

As admitted by the Examiner (Examiner's Answer, page 4, 2nd paragraph) and at least for the reasons set forth above with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of claim 6.

Claim 7

Claim 7 is Nonobvious In View of the Prior Art

As admitted by the Examiner (Examiner's Answer, page 4, 2nd paragraph) and at least for the reasons set forth above with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of claim 7. Skraly specifically states "we have also developed novel processes for the production of 3HV and 3HP containing PHAs from 1,2-propanediol and glycerol. These systems use glycerol dehydrates to generate the intermediate aldehyde that is converted to the hydroxyacyl-CoA" (see Skraly "Conclusion"). This is a different system from the claimed method. There is no reason why one of ordinary skill in the art would *omit* glycerol dehydratase from the pathway disclosed in Skraly or add genetically engineered aldehyde dehydrogenase.

Claim 8

Claim 8 is Nonobvious In View of the Prior Art

For at least for the reasons set forth above with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of claim 8.

Claim 9

Claim 9 is Nonobvious In View of the Prior Art

Claim 9 depends from claim 8 and further defines the organism as selected from the group consisting of *Escherichia coli*, *Ralstonia eutropha*, *Klebsiella* spp., *Alcaligenes latus*, *Azotobacter* spp., and *Comamonas* spp. For at least the reasons set forth with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of the claim.

Claim 10

Claim 10 is Nonobvious In View of the Prior Art

For at least for the reasons set forth above with respect to claim 1, the combination of the cited references fails to disclose or suggest each element of claim 10.

Conclusion

The Examiner has not established a *prima facie* case of obviousness. The references in combination do not recite the elements of the claims. In analyzing Skraly, the Examiner has repeatedly mischaracterized the disclosure in Skraly:

- Skraly discloses a method for making PHAs using 1,2-propanediol (a diol) in *E. coli* that requires glycerol dehydratase and exogenous coenzyme B-12; the claimed method does not require these two elements.
- Skraly does not disclose genetically engineering any organism to express enzymes selected from the group consisting of aldehyde dehydrogenase and diol oxidoreductase.
- There is no reason why one of ordinary skill in the art would modify the pathway used to generate 4-hydroxybutyrate from 1,4-butanediol and 5-hydroxyvalerate from 1,5-pentandediol (Skraly, page 7) to include a diol oxidoreductase and omit glycerol dehydratase; such modification however, would not recite all of the claim limitations or produce the unexpected results obtained with the claimed method.
- Appellants have demonstrated unexpected results over a combination of the prior art.

Therefore, even if the examiner had made a *prima facie* case, the evidence before this Board demonstrates unexpected results, including at least the higher yield and not having to use all of the enzymes that are required by the prior art to produce PHAs from a cheap source of diols, which rebuts the alleged obviousness.

Respectfully submitted,

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